

# POTASSIUM OXALATE AS A NITRIFICATION INHIBITOR AND ITS EFFECT ON MICROBIAL POPULATIONS AND ACTIVITIES IN A CALCAREOUS UZBEKISTANIAN SOIL UNDER COTTON CULTIVATION

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## ABSTRACT

Application of fertilizers combined with nitrification inhibitors affects soil microbial biomass and activity. The objective of this research was to determine the effects of fertilizer application combined with the nitrification inhibitor potassium oxalate (PO) on soil microbial population and activities in a nitrogen poor soil in Uzbekistan under cotton cultivation. Fertilizer treatments were N as urea, P as monoammonium phosphate (MAP), and K as potassium chloride. The nitrification inhibitor, (PO) was added to urea and MAP at the rate of 2%. Two treatments: N<sub>150</sub>P<sub>140</sub>K<sub>60</sub> (T1) and N<sub>150</sub>PO<sub>140</sub>K<sub>60</sub> (T2) (subscripts are concentrations in mg kg<sup>-1</sup> soil) were applied. The control (C) was without fertilizer and PO. Populations of oligotrophic bacteria, ammonifying bacteria, nitrifying bacteria, denitrifying bacteria, mineral assimilating bacteria, oligonitrophilic bacteria, and *Azotobacter* were determined by the most probable number method. Treatment T2 increased the number of oligonitrophilic bacteria, utilization of mineral forms of nitrogen by ammonifying bacteria, decreased the number of nitrifying bacteria, denitrifying bacteria and net nitrification, and increased the cellulose degradation activity of soil. In conclusion, our experiment showed that PO combined with mineral fertilizer is a most promising compound for inhibiting nitrification, which increased N fertilizer availability and efficiency to the cotton plants.

## KEYWORDS

Urea, MAP, nitrification inhibitor, cellulose decomposition, microorganisms

## INTRODUCTION

Nitrogen fertilizers in the form of urea are commonly applied in Uzbekistan in order to increase cotton yield in low fertile soils. The NO<sub>3</sub> formed through nitrification of

urea is susceptible to loss by leaching and may contribute to NO<sub>3</sub> pollution of ground- and surface waters. Treatments of fertilizers with nitrification inhibitors have been suggested as a technique to reduce the nitrification rate and NH<sub>3</sub> volatilization (Malzer, 1979; Malhi and Nylorg, 1982; McCarty and Bremner, 1990; Freney *et al.*, 1992). Nitrification inhibitors may potentially reduce NO<sub>3</sub> losses by leaching from NH<sub>4</sub>-N, liberating fertilizer materials, including organic N sources, by maintaining N as NH<sub>4</sub><sup>+</sup>, which is less susceptible to loss from the soil by this route, and NH<sub>3</sub> volatilization (Bremner and Krogmeier, 1989; Smith and Hadley, 1992; Poberejskaya *et al.*, 1993; Kholdebarin *et al.*, 1998). The soil microorganisms are thus of great importance to the nitrogen nutrition of the crop vegetation. They are sensitive to changes in the surrounding soil (Hodges, 1990; Schinner and Sonnletner, 1996). It has been shown that the microbial population changes after fertilization (Hyman *et al.*, 1990; Dobbs, 1992; Anonymous, 1992). Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil (Khonje *et al.*, 1989; Sarathchandra *et al.*, 1989; Khamis *et al.*, 1990).

The effects of the nitrification inhibitor, Potassium oxalate, with a combination of fertilizers (200kg ha<sup>-1</sup>) on the soil microbial population has been studied in our previous work experiments. In this study we reduced the amount of fertilizer combined with Potassium oxalate. The purpose of this study was to investigate the influence of mineral fertilizer (150 kg ha<sup>-1</sup>) combined with potassium oxalate on the soil microbial population and the activities and nitrification rate in nitrogen deficient calcareous soil Uzbekistan under cotton cultivation.

## MATERIALS AND METHODS

### STUDY SITE AND SOIL SAMPLING

Sites used in this study represent continuously cultivated (more than 50 years) cotton fields located in Kalinin province, the northeastern part of Uzbekistan. The soil type is calcareous Calcisol having a calcic horizon within 80 cm of the surface. The orchic horizon is low in organic matter. The climate is semi arid with mean annual air temperatures of 16°C and 18°C, and mean annual rainfalls of 200 mm. Soil samples were taken from the top 10 cm of soil from an existing cotton field. The cores were pooled and field-moist soils were sieved (<2mm) directly after collection. The soil samples were kept in black polyethylene bags and stored at 4°C. These "fresh" field-moist, sieved samples were used for the incubation study.

### POT EXPERIMENTS

Soil microbial activity and N transformation in soils amended with the mineral fertilizers and combined with potassium oxalate were studied in small pots in laboratory experiments with three replicates. Field-moist sub samples (1kg) of each treatment replicate were placed in pots and treated with N as Urea at a rate of 150 mg kg<sup>-1</sup> soil. P was supplied as MAP at a rate of 140 mg P kg<sup>-1</sup> soil and Potassium chloride at a rate of 60 mg K kg<sup>-1</sup> soil. PO was added to urea and MAP at a rate of 2%. The control pots (N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>) received neither PO nor fertilizer. Two treatments: N<sub>150</sub>P<sub>140</sub>K<sub>60</sub> (T1) and N<sub>150PO</sub>P<sub>140</sub>K<sub>60</sub> (T2) were applied for this study. The tested pots were then placed in incubators maintained at 27°C for 45 days.

### SOIL CHEMICAL AND PHYSICAL ANALYSIS

Air-dried samples were analyzed for the total C, N, P, K and Mg contents. Soil particle distribution was determined using sodium phosphate. The soil chemical and physical properties are presented in Table 1. The total carbon content, C<sub>tot</sub>, was identified by elementary analysis while total nitrogen, N<sub>tot</sub>, content was determined by the Kjeldahl method. The molybdenum blue method determined the total phosphorus content, P<sub>tot</sub>, in soil. Potassium, K, was determined using the Flame Photometric Method (Riehm, 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chlorite (CaCl<sub>2</sub>) and extractable magnesium (Schachtachnabel and Heinemann, 1974). Soil pH-value was measured by means of electrometer.

### SOIL MICROBIOLOGICAL ANALYSES

After 45 days pots were removed from the incubation and were analysed for microbiologic tests. A plate dilution method

was used for the determination of numerous microorganisms using agar medium. In order to count the number of microorganisms, 10 g of soil was shaken with 90 ml of ster-distilled water. From this suspension the serial dilution (1:10) was prepared and plate counts were performed in triplate and incubated until growth occurred (usually 3-7 days). CFU of ammonifying bacteria were enumerated on glycerine peptone agar. Mediums containing 10 g of starch, 2 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g of K<sub>2</sub>HPO<sub>4</sub>, 1 g of MgSO<sub>4</sub> and 3 g CaCO<sub>3</sub>, 1 g of NaCl and 15 g of agar l<sup>-1</sup> were used for mineral assimilating bacteria. Nitrifying bacteria were determined on plates containing 2 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 g of K<sub>2</sub>HPO<sub>4</sub>, 0.5 g of MgSO<sub>4</sub>, 0.1 g FeSO<sub>4</sub>, 5 g CaCO<sub>3</sub>, and 0.4 g NaCl l<sup>-1</sup> of liquid medium. Denitrifying bacteria on Giltay medium containing 1 g KNO<sub>3</sub>, 1g KH<sub>2</sub>PO<sub>4</sub>, 1g K<sub>2</sub>HPO<sub>4</sub>, 2 g MgSO<sub>4</sub>, 0.2 g CaCl<sub>2</sub>, 0.1 mg FeCl<sub>3</sub>, 0.1% solution of brom thimolblue, oligotrophic bacteria on soil agar containing 900 ml water, 100g soil, 18g agar L<sup>-1</sup>, oligonitrophilic bacteria and *Azotobacter* were determined on Eshbi agar containing 0.2 g K<sub>2</sub>HPO<sub>4</sub>, 0.2 g MgSO<sub>4</sub>, 0.2 g of NaCl, 0.1 g K<sub>2</sub>SO<sub>4</sub>, 5 g CaCl<sub>2</sub>, 20 g sacharosa, and agar 15 g l<sup>-1</sup>.

### SOIL BIOCHEMICAL MEASUREMENTS

Cellulose degrading activity of soil was measured according to Swyaginzew (1987). Cellulose material was placed into soil for an incubation period of 45 days. After 45 days the material was removed and the cellulose degradation percentage was analyzed. Net nitrifications were measured by incubating the soil samples with the soil moisture content adjusted to 60% of the WHC at 28°C for 45 days. The method used is described in detail in Aristowskaya (1962). The data were analyzed using the statistical analysis of variance by Tepper (1974).

## RESULTS AND DISCUSSION

### CHANGES IN SOIL MICROBIAL POPULATIONS

T1 and T2 decreased the number of oligotrophic bacteria compared to the control (Table 2). A decreasing of coloniza-

**Table 1.** Chemical and physical parameters for the 0-30cm depth.

|                                   |                  | Chemical |    |    |               | Physical |      |      |
|-----------------------------------|------------------|----------|----|----|---------------|----------|------|------|
| C <sub>tot</sub>                  | N <sub>tot</sub> | P        | K  | Mg | pH            | Sand     | Silt | Clay |
| ----- mg 100g <sup>-1</sup> ----- |                  |          |    |    | ----- % ----- |          |      |      |
| 200                               | .6               | 3        | 12 | 6  | 8.5           | 2.2      | 54.5 | 9.4  |

tion frequency of oligotrophic bacteria after inhibitor nitrification in cotton plants has been also reported (Poberejskaya *et al.*, 1993). Oligotrophic microorganisms are able to survive in low nutrient content soil. An input of a high concentration of nutrients inhibited their activity. The T1

bacteria compared to the control (Table 3). The results showed that PO inhibited nitrifies, which was reflected in the reduced  $\text{NO}_3^-$  losses by leaching from fertilizer material. Other authors also reported that Thiourea inhibited the nitrifying activity of nitrifies, which reflected in the increased availability and efficiency of fertilizer nitrogen to the rice plants and indicated a potential as a nitrification inhibitor (Fog, 1988; Witthaya and Thongpan, 1987).

**Table 2.** Effect of mineral fertilizer combined with potassium oxalate (PO) on the number of oligotrophic, ammonifying and mineral assimilating bacteria ( $10^6\text{cfu g}^{-1}$  soil)

| Treatment                                      | Oligo-trophic    | Ammonifying    | Mineral assimilating |
|--|------------------|----------------|----------------------|
| $\text{N}_0\text{P}_0\text{K}_0$               | $184.0 \pm 0.62$ | $7.7 \pm 0.25$ | $94.7 \pm 0.59$      |
| $\text{N}_{150}\text{P}_{140}\text{K}_{60}$    | $10.6 \pm 0.51$  | $8.9 \pm 0.55$ | $27.4 \pm 0.62$      |
| $\text{N}_{150}\text{PO P}_{140}\text{K}_{60}$ | $50.0 \pm 0.77$  | $4.5 \pm 0.47$ | $17.2 \pm 0.83$      |

and T2 decreased the number of mineral assimilating bacteria (Table 2). In particular, decreasing soil water potential following mineral N application and declining pH resulting from nitrification of  $\text{NH}_4^+$  sources are known to reduce the activity of mineral assimilating microorganisms (Soderström *et al.*, 1983).

The number of ammonifying bacteria were reduced by T2 (Table 2). That shows the utilization of mineral forms of nitrogen in soil on the background of reducing the quantity of ammonifying bacteria. A decreased number of ammonifying bacteria after the application of the nitrification inhibitor in Calcisol soil has been reported early (Poberejskaya *et al.*, 1993). The decrease in microbial indices in the fertilizer treatments could indicate a change in the quality of organic matter to a less available substrate for ammonifying bacteria than in the no fertilized soil (Nohrsedt *et al.*, 1989). An increased population of oligonitrophilic bacteria 3-6 times compared to that of controlled was found after T1 and T2 (Table 3). All treatments had no negative effect on nitrogen fixing bacteria *Azotobacter* (data not shown). According to (Miyan *et al.*, 1986; Kucharski, 1991; Govedarica *et al.*, 1999), the treatments of nitrification inhibitors also increased the number of oligonitrophilic bacteria and had no negative effects on *Azotobacter*.

Treatments T1 and T2 decreased the number of nitrifying

The number of denitrifying bacteria with T2 significantly decreased 12 times in comparison with the control (Table 3). Denitrifying activity is an indicator of the carbon mineralization of soil. Nitrogen fertilization may result in an unbalanced nutrient composition in the soil, which can reduce the denitrifying activity of bacteria. Nitrification inhibitors have a very marked effect on production of  $\text{N}_2$  and  $\text{N}_2\text{O}$  through the reduction of  $\text{NO}_3^-$  by denitrifying microorganisms because it blocks the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  by these microorganisms (Yoshinari and Knowles, 1976). It also has been found that urea fertilization increases the pH value and results in decreased microbial biomass and activity (Nimmik and Wiklander, 1983).

**CHANGES IN BIOCHEMICAL PROPERTIES**

The application of fertilizer increased net nitrification 7 times compared to the control (Table 4). T2 decreased the net nitrification compared with fertilizer alone. Nitrification inhibitors reduced the rate of nitrification and so increased the thermal-time required for  $\text{NH}_4\text{-N}$  depletion and  $\text{NO}_3\text{-N}$  accumulation in soil amended with  $\text{NH}_4\text{-N}$  forming materials compared with fertilizer alone. Some authors suggested the reduction of nitrification after application of nitrification inhibitors Malhi and Nylorg, 1982; Hyman *et al.*, 1990; Smith and Hadley, 1992). Our results indicate that PO slows the rate of nitrification and may effectively reduce potential  $\text{NO}_3^-$  leaching losses.

To assess the potential value of a PO in soil, it is important to have information concerning other trials' formations of N in soil. The study of the effect of PO on cellulose degradation activity in soil showed that T1 and T2 increased the cellulose degradation activity of soil, which shows the increasing number of cellulose degrading microorganisms (Table 4). Other authors also found that after application of mineral fertilizers, the number of cellulolytic microorganisms became higher (Govedarica *et al.*, 1999).

**Table 3.** Effect of mineral fertilizer combined with potassium oxalate (PO) on the number of oligonitrophilic denitrifying and nitrifying bacteria ( $10^6\text{cfu g}^{-1}$  soil)

| Treatment                                      | Oligonitrophilic | Denitrifying     | Nitrifying       |
|--|------------------|------------------|------------------|
| $\text{N}_0\text{P}_0\text{K}_0$               | $2.6 \pm 0.90$   | $700.0 \pm 0.59$ | $0.110 \pm 0.79$ |
| $\text{N}_{150}\text{P}_{140}\text{K}_{60}$    | $12.7 \pm 0.67$  | $70.0 \pm 0.65$  | $0.25 \pm 0.49$  |
| $\text{N}_{150}\text{PO P}_{140}\text{K}_{60}$ | $7.3 \pm 0.59$   | $60.0 \pm 0.38$  | $0.13 \pm 0.55$  |

**Table 4.** Effect of mineral fertilizer combined with Potassium oxalate (PO) on the net nitrification and cellulose degradation activity of soil.

| Treatment  | Net nitrification<br>mg N-NO <sub>3</sub><br>100g <sup>-1</sup> soil | Cellulose degradation<br>----- % ----- |
|--|--|--|
| N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>         | 2.15 ± 0.72  | 0.8 ± 0.85                             |
| N <sub>150</sub> P <sub>140</sub> K <sub>60</sub>    | 12.15 ± 1.14   | 12.3 ± 1.04                            |
| N <sub>150</sub> PO P <sub>140</sub> K <sub>60</sub> | 6.98 ± 1.00  | 3.75 ± 1.98                            |

## CONCLUSIONS

It was clearly demonstrated that fertilization supplied with nitrification inhibitors influenced soil microorganisms. All combinations of mineral fertilizers combined with PO during the incubation had an inhibitory effect on the activity of oligotrophic bacteria, ammonifying bacteria, and denitrifying bacteria. The marked stimulus effect on the number of bacteria during the incubation was achieved with T2 and the lowest with T1. To summarize, the work reported in this paper suggests that PO combined with mineral fertilizers had no adverse effects on the biological nitrogen fixing bacteria *Azotobacter* and increased the activity of oligonitrophilic bacteria and increased the cellulose degrading activity in soil. PO indicated potential as nitrification inhibitors for the soil of urea used in this study. The treatment T2 decreased the net nitrification compared with fertilizer alone. In conclusion PO is one of the promising nitrification inhibitor compounds for reducing potential NO<sub>3</sub><sup>-</sup> leaching losses by nitrifying microorganisms from materials during cotton plant establishment.

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